particular
localization of gene and messages contained their in.

Immunohistochemistry: In place of probe, we can use antibody.

2010

POLYMERASE CHAIN REACTION

As the name indicates, this rkn uses polymerase enzyme chain. Here, when DNA is duplicated, the separate strand act as template and replication process is again repeated.

Historical Approach:-

Idea was given in 1970s

This rkn is sensitive to temperature changes.

Around 1975 - 1980s enzyme Taq polymerase was discovered from a thermophilic bacteria Thermus aquaticus This bacteria is usually found in hot springs (60-70°c temp. range). In place of DNA polymerase; this Taq polymerase is used.

Kary Mullis (USA in Cetus corporation of California) first performed PCR and got amplified DNA.

Thermal cyclers can perform quick heating and cooling which facilitate PCR.

In 1983 it was discovered.

1983 & 1993 Kary Mullis got Nobel Prize for discovery of PCR in Chemistry.

Peltier system: Efficient cooling and heating (quickly) could be done by this method by reversing the current direction.

In case of PCR we copy the DNA and then paste it.

day

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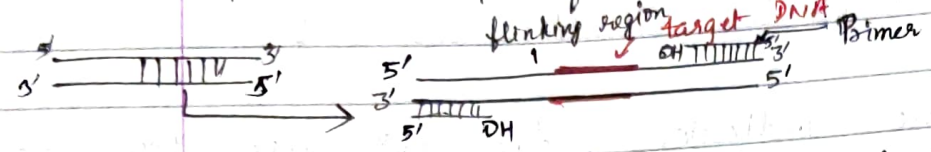
✱ PROCESS:

Template of target DNA is taken (other parts of DNA could also be amplified).
- Upto 10 Kbp of DNA amplification could be done.



Replication need a small oligonucleotide (20 nt long) is called primer. This primer can bind with flanking region of DNA of desired seq. i.e. target DNA.

→ dNTPs – deoxy nucleotide triphosphate.
Taq polymerase
buffer
Mg^{2+}
KCl

all component is putted in tube ▽ 0.2 - 0.5 ml (20 - 200 µl) thin walled microtubes

⚠ any defect will block PCR.

~~Thermal cycle~~: Put tube in thermal cycle block and covers it by hot lid.
- The rxn starts and first step of rxn is
(1) denaturation DNA (two strands are separated) (20-30 sec)
(2) Annealing (due to complementarity bases will be joined at low temperature) 65°C

Melting temp. is given by formula.

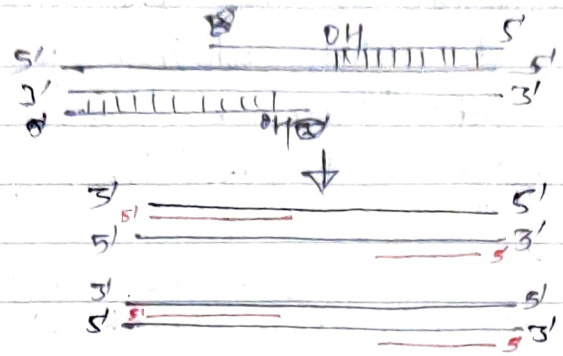
$$Tm = (2(A+T) + 4(G+C))^{\circ}C$$

±5°C ±2°C

Annealing temp is 5°C lower than Tm.

GD C

(3) Elongation - Polymerisation occure at 70-75°c and strands elongated.



This replication is exponential in terms of 2^n. We can take amplified millions of molecules

These 3 steps are repeated again and again.

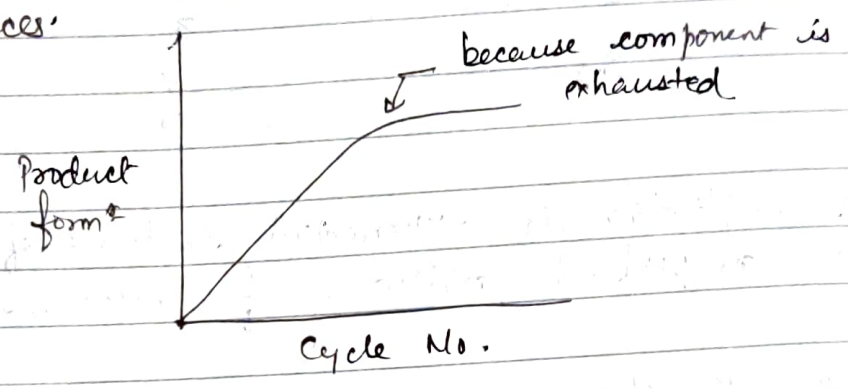
(4) Hold - At the end of cycle, elongation is performed at 72°C for 5 min. This is c/a hold.

- The last step is to hold 2^n at 4-5°C.
This is a kind of automated thermal cycle.
- We cand do 2% of Agarose Gel Electrophoreses of PCR product or amplicon and can calculate the size. If size is same throughout then we can say that PCR is performed errorfreely

Taq polymerase usually causes 10^{-3} to 10^{-6} errors. So one error is incorporated per 10^{-3} - 10^{-6} nucleotides.
This sequencing is performed.

During elongation 1000 nucleotides are added per minute.

- for unk/n sequences, we use universal primer we have to do trial whether primer is get annealed or not. There are several techniques which can design primers for specific unknown sequences.



All the sample (initials) should be in excess if once exhaustation occure, PCR will be interrupted. All materials should be optimised.

Applications of PCR:

(1) DNA can be amplified.
(2.) For sequencing DNA — Sanger method.
(3) DNA phylogeny, taxonomy and evolutionary significance could be identified.
(4) Expression profile — There are diff. modifcation which could be known by this.
(5) RT-PCR — Reverse transcription is done with help of RTase and RNA will give cDNA by this method and by this method cDNA is amplified.

RNA $\xrightarrow{RT}$ cDNA $\xrightarrow{PCR}$ amplification.

(6) SNPs — Single Nuclear Polymorphs determination.